(2) H-Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His-OH;

(3) Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His;

(4) His Pro Leu Gly Ser Pro Gly Ser Ala Ser Asp Leu Glu Thr Ser Gly Leu Gln Glu Gln Arg Asn His Leu Gln Gly Lys Leu Ser Glu Leu Gln Val Glu Gln Thr Ser Leu Glu Pro Leu Gln Glu Ser Pro Arg Pro Thr Gly Val Trp Lys Ser Arg Glu Val Ala Thr Glu Gly Ile Arg Gly His Arg Lys Met Val Leu Tyr Thr Leu Arg Ala Pro Arg Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu; and

(5) Met Asp Pro Gln Thr Ala Pro Ser Arg Ala Leu Leu Leu Leu Leu Phe Leu His Leu Ala Phe Leu Gly Gly Arg Ser His Pro Leu Gly Ser Pro Gly Ser Ala Ser Asp Leu Glu Thr Ser Gly Led Gln Glu Gln Arg Asn His Leu Gln Gly Lys Leu Ser Glu Leu Gln Val Glu Gln Thr Ser Leu Glu Pro Leu Gln Glu Ser Pro Arg Pro Thr Gly Val Trp Lys Ser Arg Glu Val Ala Thr Glu Gly Ile Arg Gly His Arg Lys Met Val Leu Tyr Thr Leu Arg Ala Pro Arg Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His.

The present invention also concerns a recombinant DNA sequence comprising a base sequence encoding a polypeptide

having one of the amino acid sequences (1)-(5) above, as well as a method of producing cDNA, comprising:

hybridizing a probe having a DNA sequence encoding a part of porcine brain natriuretic peptide to a human cDNA library;

selecting a positive clone; and

isolating said cDNA of said positive clone.

As demonstrated by the factual evidence and statements in the accompanying (unexecuted) Declaration, one of ordinary skill would not have had a reasonable expectation of success in arriving at the present invention from the knowledge present in the art at the time grandparent application Serial No. 07/486,827 was filed (March 1, 1990). (The executed Declaration will be filed as soon as Applicants' U.S. representative receives the same.)

For example, neither the 70% degree of homology between human atrial natriuretic peptide (hANP) and porcine BNP (pBNP) taught by Sudoh et al (*Biochem. Biophys. Res. Comm.*, 155:726-732 and *Nature*, 332:78-80) nor the 50.6-65.7% degree of homology between hANP mRNA and pBNP mRNA taught by Maekawa et al is sufficiently high for one of ordinary skill to reasonably expect success in cloning and isolating the cDNA of one based on the sequence of the other (see paragraph 5, page 3 of the accompanying Declaration).

Further, Table 1 of Oikawa et al teaches that the homology between hANP and other mammalian ANPs is only 52-60%. Thus, assuming that one of ordinary skill expects the same

degree of homology between hBNP and other mammalian BNPs as is observed between hANP and other mammalian ANPs, Sudoh et al (*Biochem. Biophys. Res. Comm.*, 155:726-732 and *Nature*, 332:78-80), Maekawa et al and Oikawa et al appear to indicate that the degree of homology is greater between pBNP and hANP than what one expects between pBNP and hBNP. As a result, one might expect a probe based on the pBNP gene to lead to cloning of a hANP gene, rather than a hBNP gene (see paragraph 6, pages 3-4 of the accompanying Declaration).

In addition, Sudoh et al (*Biochem. Biophys. Res. Comm.*, 159:1427-1434, attached to and incorporated into the Declaration by reference) disclose that human and porcine ANP's have 89.7% and 100% identical residues in the pro-form and α-form, respectively (page 1433, lines 1-3). However, the high homology between the pro- and α-forms of hANP and pANP would lead one to reasonable expect success in cloning and isolating hBNP cDNA using a 10-20 bp pBNP probe, which the present Inventors attempted but failed to successfully carry out (see paragraph 7, page 4 of the accompanying Declaration).

Furthermore, the low homology (70.0%) between human prepro-BNP and porcine prepro-BNP (results determined by the present Inventors, disclosed by Sudoh et al [*Biochem. Biophys. Res. Comm.*, 159:1427-1434]) presents a sharp contrast to the more highly conserved mammalian ANP's, thus introducing an unexpected problem in cloning hBNP. This unexpected problem makes it surprising that hBNP cDNA could be cloned and isolated, given the level of ordinary skill and the knowledge

in the art at the time of filing grandparent U.S. application Serial No. 07/486,827 (March 1, 1990) (see paragraph 9, page 4 of the accompanying Declaration). Therefore, one of ordinary skill would not have had a reasonable expectation of success in arriving at the present invention from the knowledge present in the art at the time grandparent application Serial No. 07/486,827 was filed.

Furthermore, as discussed in the Amendment filed July 21, 1993 and the Preliminary Amendment filed February 7, 1994, Seilhamer et al, U.S. Patent No. 5,114,923, appear to teach away from the manner in which the present Inventors proceeded in obtaining the presently claimed DNA sequences. In view of the failure of Seilhamer et al to hybridize DNA encoding pBNP to human DNA, the success of the present Inventors in obtaining DNA encoding human BNP is surprising and unexpected.

Even further, the present invention provides a means for obtaining a human natriuretic peptide which is expected to have considerably higher rectum relaxation activity than the corresponding ANP, based on the known 3- to 4-fold increase in the rectum relaxation activity of pBNP relative to pANP (see page 2, lines 3-15 of the present specification). Consequently, the present invention represents a significant and meaningful advancement in the art.

The rejection of Claims 2-7 and 10-23 under 35 U.S.C. 103 as being unpatentable over Maekawa et al in view of Maniatis, Sudoh et al, (R) and Sudoh et al (T), Oikawa et al and Vlasuk et al is respectfully traversed.

Maekawa et al disclose cloning and sequence analysis of cDNA encoding a precursor for porcine brain natriuretic peptide (pBNP). Sudoh et al (R) disclose the sequences of porcine BNP and human α-ANP (atrial natriuretic peptide). Sudoh et al (T) disclose a 32-amino acid-long brain natriuretic peptide identified in porcine brain. Oikawa et al disclose the structure of dog and rabbit precursors of atrial natriuretic peptides deduced from nucleotide sequences of cloned cDNA. Vlasuk et al disclose the structure and analysis of the bovine atrial natriuretic peptide precursor gene.

Maniatis et al teach the expression of eucaryotic genes, vectors that express fused and unfused eucaryotic proteins, and synthetic oligodeoxynucleotides useful for hybridizing to (and screening) mRNA. The method of screening MRNA disclosed by Maniatis et al is essentially the method disclosed by Suggs et al, cited against the present invention by the Examiner in parent application Serial No. 07/486,827, and withdrawn by the Examiner in the present application. Similar to the teachings of Suggs et al, one must scan the known protein sequence for areas rich in amino acids specified by one or two codons (page 226, lines 3-6 from the bottom). The oligonucleotides of Maniatis et al, similar to those of Suggs et al, form hybrids only with those species of mRNA to which they are exactly complementary (page 227, lines 4-7).

Therefore, although Maniatis et al suggest that their oligodeoxynucleotides can be used as probes to screen cDNA libraries, the teachings of Maniatis et al fail to cure the

deficiencies of the remaining of the cited references. The method of Maniatis et al requires that the amino acid sequence be known. Prior to the present invention, however, the amino acid sequence of human BNP was not known. Further, without the absolute assurance that there will be at least one probe which is exactly complementary to the target sequence provided by knowledge of the amino acid sequence, success cannot be achieved. A reasonable expectation of success using the method of Maniatis et al cannot exist when the amino acid sequence encoded by the target sequence is not known.

However, assuming *arguendo* that it would have been obvious for one of ordinary skill in the art to use the porcine DNA sequence disclosed by Maekawa et al, or an effective portion thereof (as taught by Seilhamer et al [col. 8, lines 40-54]), as a probe to screen for the human BNP gene, the factual bases by which one of ordinary skill evaluates the expectation of success as set forth in the accompanying Declaration support the patentability of the present invention.

Furthermore, the clear failure of Seilhamer et al to successfully achieve this result also attests to the nonobviousness of the present DNA. Interestingly, as discussed above, Seilhamer et al equate a genomic library with a cDNA library, thereby indicating that no unusual problems are expected in screening a genomic library. Even more interesting, the temperature conditions used by Seilhamer et al were less stringent (37-42°C; col. 9, lines 6-10 and 38-42)

than those used to obtain the present DNA sequences (60°C; page 14, line 19 of the present specification).

Therefore, the combined teachings of the cited references do not overcome the evidence of the difficulties encountered in obtaining the present invention as described in the accompanying Declaration and by Seilhamer et al. Therefore, this ground of rejection is unsustainable, and should be withdrawn.

Accordingly, the present application is in condition for allowance. Early notice to that effect is earnestly solicited.

Respectfully submitted,

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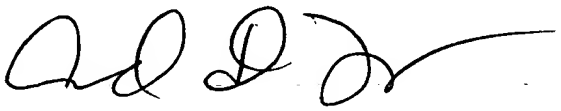

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